NSL 64-29-7

PROGRESS REPORT

INVESTIGATION OF PEROGNATHUS AS AN EXPERIMENTAL ORGANISM FOR RESEARCH IN SPACE BIOLOGY

1 April through 30 June 1965

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PREPARED UNDER CONTRACT NASw-812

for

OFFICE OF SPACE SCIENCES
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Investigation of Perognathus as an Experimental Organism for Research in Space Biology (Contract NASw-812)

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SUMMARY OF ACTIVITIES

The following summarizes progress on Contract NASw-812 between 1 April and 30 June 1965.

Three aspects of our pocket mouse program were vigorously pursued during this period: 1) studies on the mechanisms of radiation resistance; 2) establishment of a breeding colony; and 3) publication of research accomplishments to date.

Radiobiology

Pocket mouse intestinal epithelium is being closely scrutinized for clues to mechanisms of radiation resistance. As reported earlier, evidence suggests that study of cell population dynamics of the intestinal mucosa may be extremely important to the ultimate solution of this problem. Mitotic index of intestinal crypt cells, villus transit of epithelial cells, crypt and villus geometry and histology of the normal pocket mouse are being investigated. Tritiated thymidine studies are underway to elucidate certain aspects of cell population dynamics. In addition to these studies of the normal animal, some data has been collected on early post-irradiation histopathology of the intestine. Although many meaningful results are available, reporting at this time would be premature.

Breeding

Enough has been accomplished on the breeding of *Perognathus* to warrant publication. The research summarized herein, combined with related work reported in the last quarter, will serve as a basis for a paper on *Perognathus* breeding which will be submitted for publication.

Publication

A paper entitled "Mechanisms of Radiation Resistance in Pocket Mice" has been accepted for publication in *Radiation Research*.

Another paper entitled "Radiobiological Studies on *Perognathus*, a Small Desert Rodent", was presented before the Late Effects Group Colloquium at the Radiation Research Society meeting in May. This paper will appear in the Transactions of that Colloquium.

LABORATORY BREEDING OF PEROGNATHUS

P. Hayden, J. J. Gambino and R. G. Lindberg

Introduction

Routine observation of a colony of Perognathus longimembris over a 5-month period has provided data useful for successful laboratory breeding of this Heteromyid.

This is a continuation of an earlier interim communication in which our limited success at producing litters was reported (1). In that report a literature review of Heteromyid breeding, and breeding techniques used in this and other laboratories were presented.

Intensive application of these techniques has resulted in 48 laboratory-conceived litters, yielding a total of 158 pocket mice to date in this laboratory. This report updates data on reproductive activity in captive animals and in field collected animals. It summarizes results of pairing reproductively active animals, and presents more definitive data on post-natal growth and development than was reported earlier (1).

Breeding Efficiency

Complete data on estrous periodicities, mating attempts and litter production are given in figures 1 and 2. These two figures are derived from two similarly selected and treated groups of 80 females each. However, they are reported below as a composite group of 160 animals.

At least one full estrous cycle was observed in 58% (93/160) of the group during the 5-month observation period (Jan. 22 - June 25, 1965). The number of estrous cycles per animal varied from a minimum of one to a maximum of 11. The animal with 11 cycles, after several unsuccessful pairings, finally became pregnant.

A total of 217 estrous females were paired with males. From these pairings 48 litters were produced, which is about one litter produced per 5 pairings ($\frac{48 \text{ litters}}{217 \text{ attempted mating}} = 0.22$ litters/attempted mating). The sex ratio of those animals weaned as of 30 June was 47:53 (30 ♀, 34 ♂).

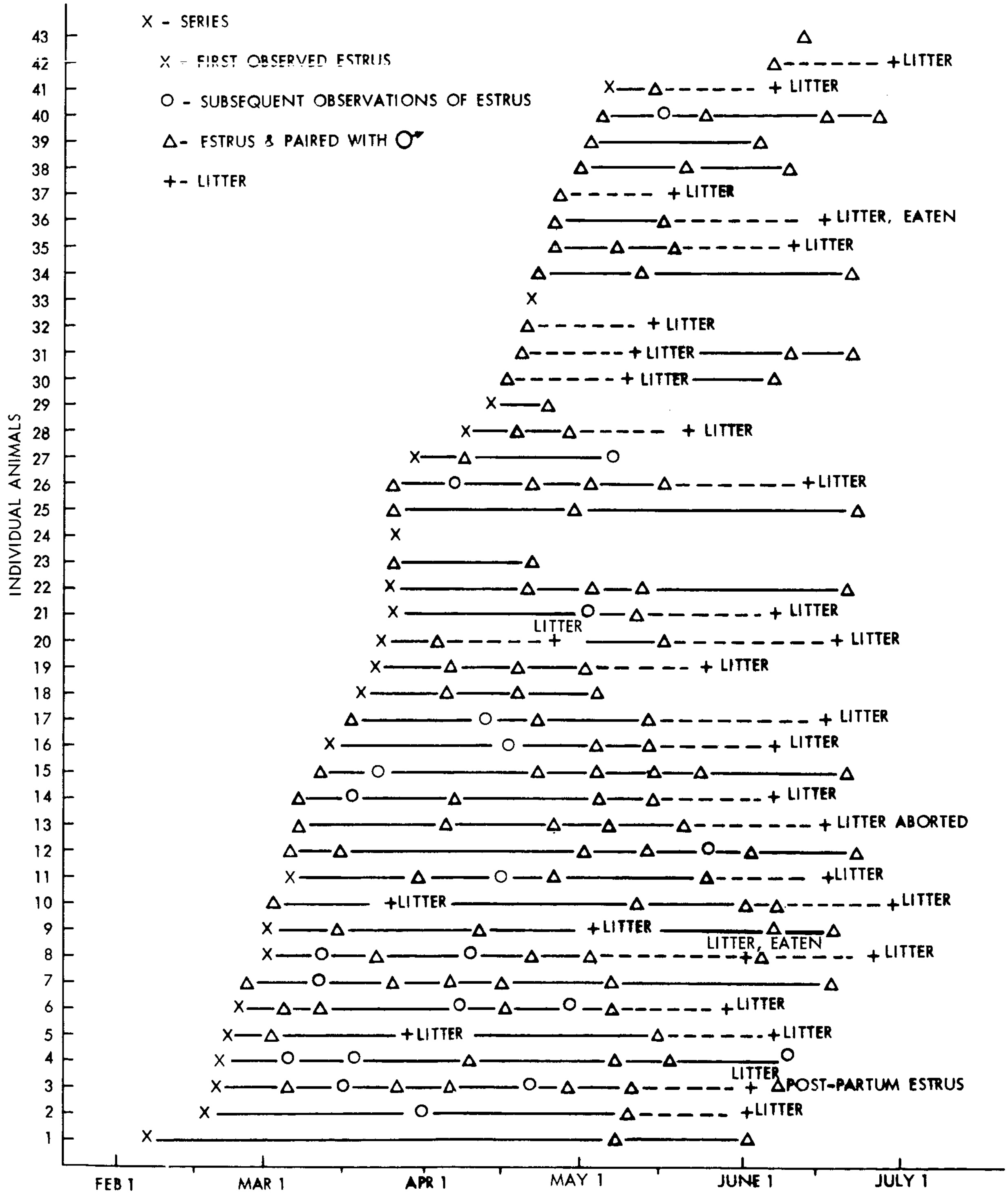


Fig. 1. Time of appearance of estrus and repeated estrous cycles in a group of 80 Perognathus longimembris

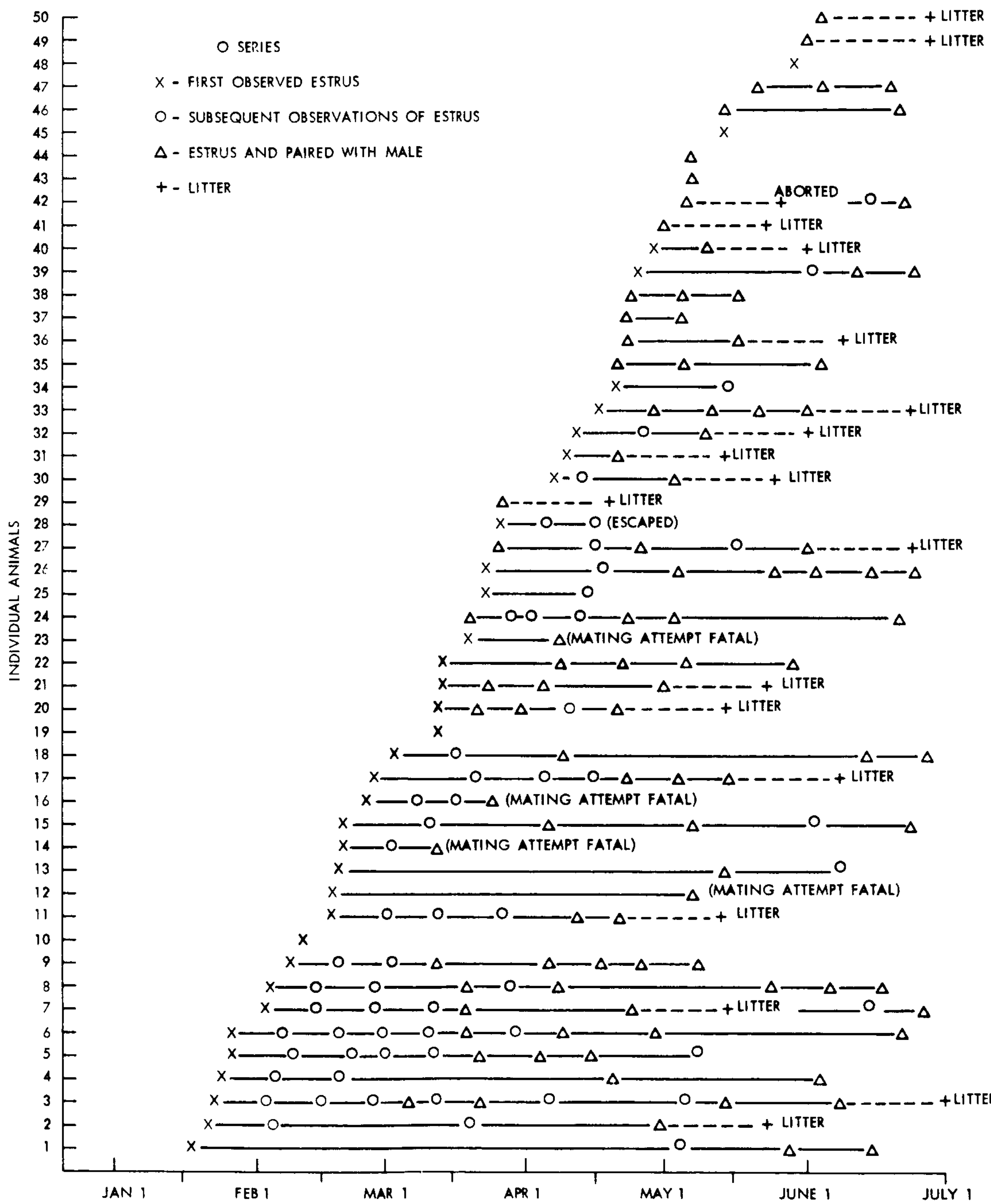


Fig. 2. Time of appearance of estrus and repeated estrous cycles in a group of 80 Perognathus longimembris

Daily inspection of reproductively active females and weekly inspection of the entire group was found most practical for collecting routine data on a large number of mice. This procedure resulted in utilizing 61% $(\frac{217 \text{ attempted mating}}{355 \text{ estrous cycles}})$ of total number of observed estrous cycles.

To be sexually receptive the female must be paired during the first half of the estrous cycle. After the vaginal lining has started to regress (i.e. appears dry and scaly) and to form the vaginal plug, the female is not receptive.

In 11 instances, the first observed estrus and pairing resulted in litter production. Two litters each have been obtained from four dams. In these cases, polyestrus was initiated no later than the third week of March.

Observed initial estrous periods, total number of estrous cycles, and number of litters are presented in table 1. There is a gradual increase of incidence of first estrus from late January to late February. A maximum number of first estrous periods occurred in March, and a gradual decline occurred in April, May and June. Since polyestrus frequently occurs, the cumulative number of estruses observed increased throughout the period from January through early May.

A total of 25 males sired the 48 litters; however, four aggressive and virile males sired 46% (22/48) of the litters. Fatalities occurred during eight attempts at mating (4 ♂, 4 ♀). The virile males all have short and severely scarred tails indicative of the pugnacious behavior of females even at a highly social time.

Field Samples of P. longimembris

Animals received from the field during the time period March 5 - June 28, 1965 are listed in table 2. The first gravid female was trapped during mid-April and juveniles began to appear approximately seven weeks later. Samples obtained during mid-June were composed mainly (84%) of juvenile animals.

Post-partum estrus

Post-parturition estrus has been documented in *P. longimembris*. In two instances females were bred and littered but cannibalized their offspring shortly after birth. The animals were subsequently returned to routine

TABLE 1. Summary of Observed Estrous Periods and Litters in 160 *Perognathus longimembris*.

Time Period	No. First Estrus		Total No. Estrous Periods	No. Litters
Jan 1 - 15	0	6	0	0
16 - 31	6		6	0
Feb 1 - 14	5	18	13	0
15 - 28	13		21	0
Mar 1 - 15	14	32	37	0
	18		42	2
Apr 1 - 15	5	23	43	0
16 - 30	18		53	2
May 1 - 15	8	11	62	8
16 - 31	3		24	9
June 1 - 15	3	3	33	14
16 - 25	0		21	13
TOTAL	93		355	48

TABLE 2. Field Collected Juvenile and Gravid Animals Received in 1965.

P. longimembris

Date	No. Received	No. Juveniles	% Juveniles	No. Gravid
5 Mar 65	7	0		0
10 Mar 65	25	0		0
17 Mar 65	6	0		0
25 Mar 65	40	0		0
19 Apr 65	23	0		1 (1 litter)
6 May 65	17	0		4 (1 litter)
17 May 65	20	0		2
2 June 65	28	7	18.4%	4 (3 litters)
8 June 65	50	26	52.0%	1
14 June 65	52	44	84.6%	2 (2 litters, 1 dead)
22 June 65	108	65	60.2%	0
28 June 65	58	30	51.7%	0

observation. Estrus was observed at two days post-parturition in one animal and at four days in the other. These females were paired again, and a litter resulted from the animal that came into estrus at 2 days post-parturition.

Juvenile estrus

In a prior report (1), observational data was presented on the occurrence of a juvenile gravid field-captured P. formosus. It was judged juvenile by coat color. We now have direct evidence that sexual maturity is attained in P. longimembris females as young as 41 days. One female of the first litter born in the laboratory was noted to have swollen external genitalia, but a sealed vaginal opening at 41 days after birth. The next day a fresh, well-formed core (sloughed vaginal lining) was observed, indicating that an estrous cycle had been completed. This individual was again noted to be entering estrus 30 days later and was paired with a male. It is still too early to determine whether impregnation occurred.

Litter growth

Data on growth of a litter from day 2 to 25 is presented in figure 3. Points are average values for four siblings (2 ♂, 2 ♀). A differential growth rate of the various parts measured is evident. Growth of ear, hind-foot and body all appear to be similar, while tail length and body weight appear to be increasing more rapidly. Plates I - III show growth and development of this species.

Nest Building

Pocket mice generally build nests when material is available. Pregnant mice in our colony were provided with dry grass for nest building, and casual observations were made on nest building behavior and nest construction.

Variation in size of nests based on amount of grass available was evident. Generally, nest building in pocket mice was reminiscent of that of birds. Pocket mice were very diligent in their work. Fine grass was cut to ½ inch lengths and used for the bulk of the nest, while coarse grass was used as bedding. The bed area was about 1½ to 2 inches in diameter. If sufficient grass was available, the nest was covered. In one instance when an excess of grass was provided, an elaborate tunnel system was made with an entrance and exit.

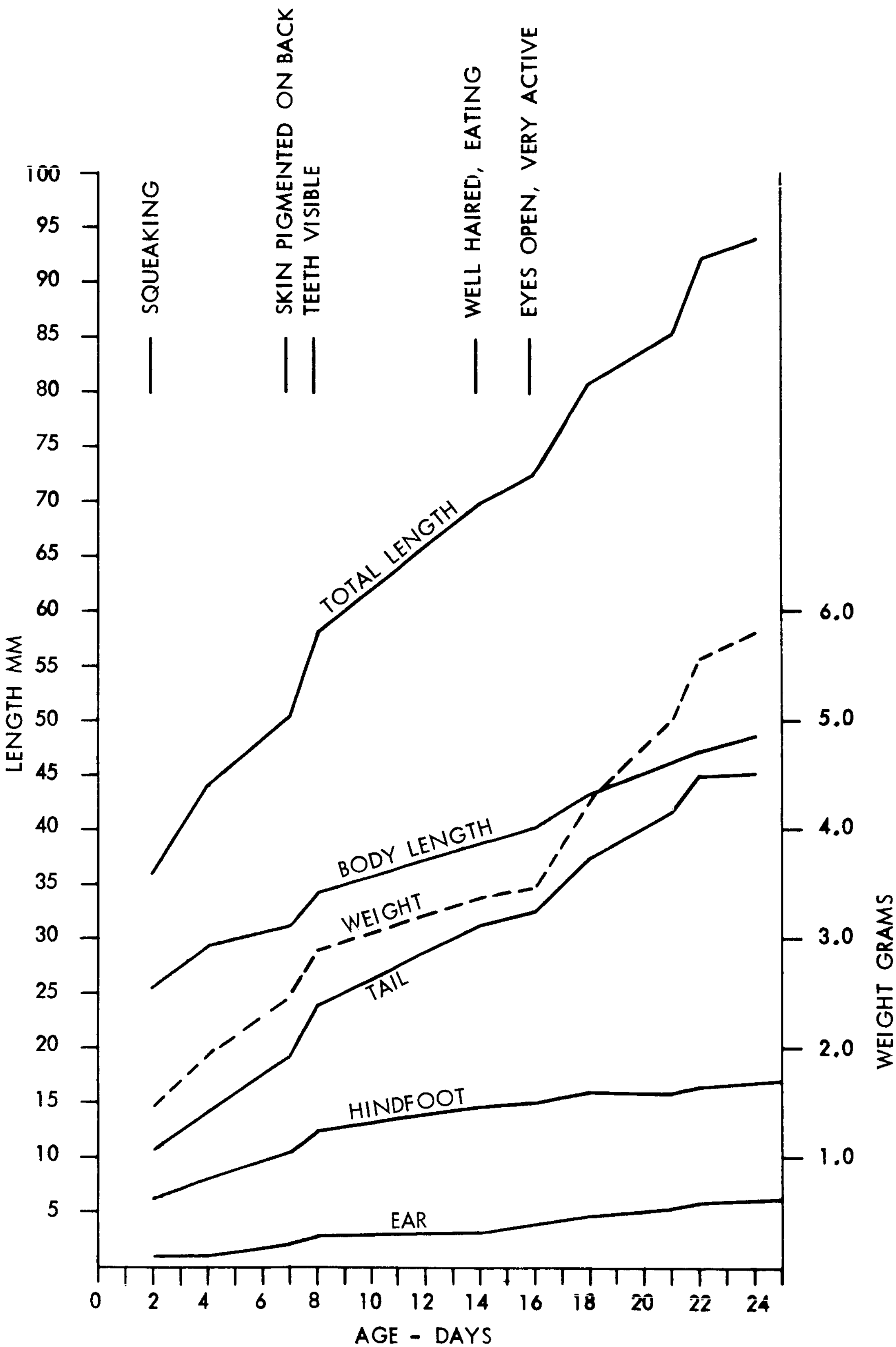
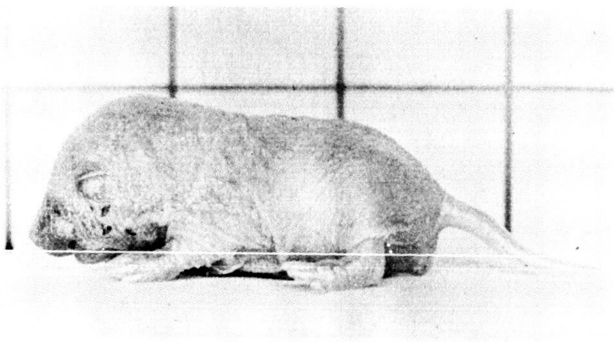
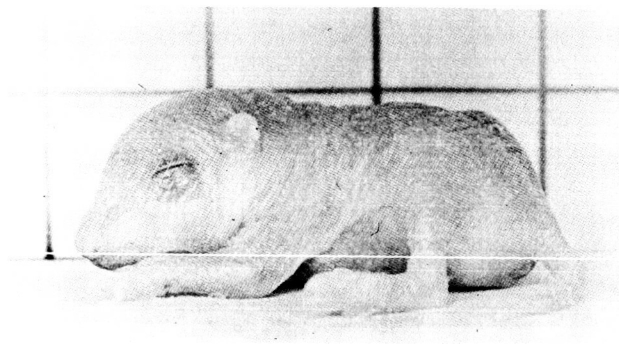


Fig. 3. Growth of Perognathus longimembris - average values for four siblings (2 ♂, 2 ♀)

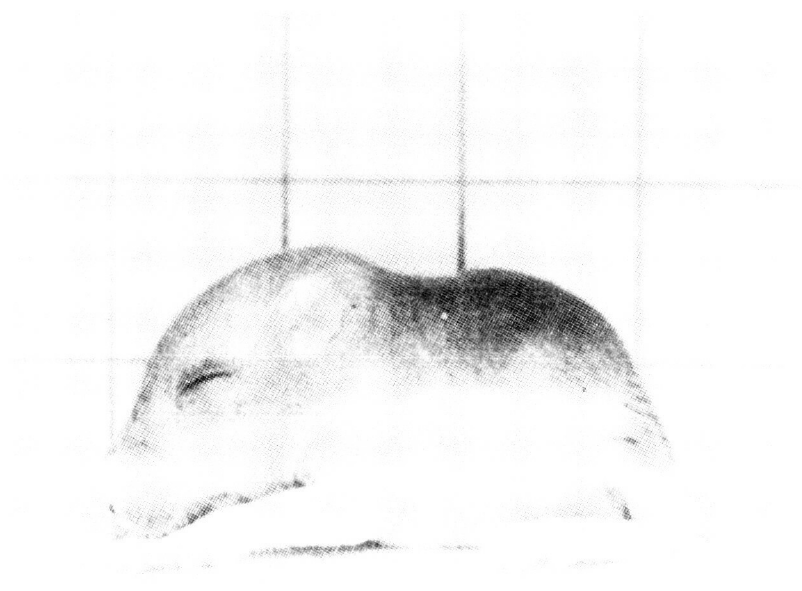


1 day



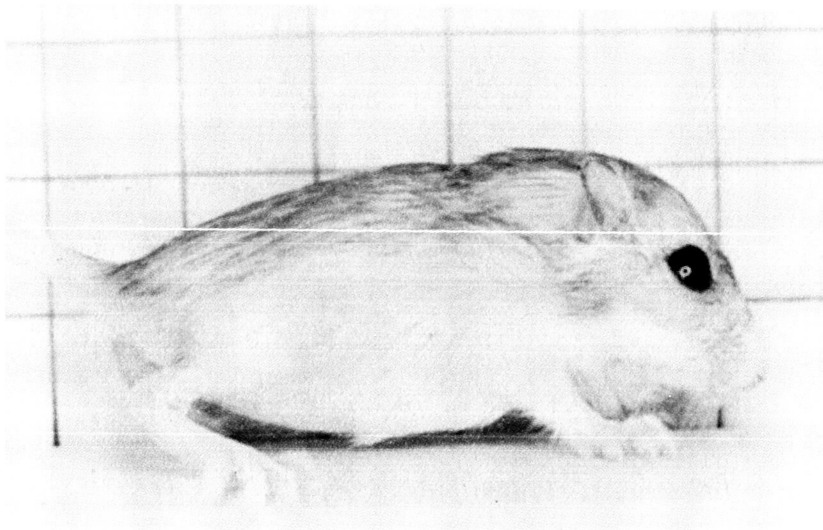
4 days

9 days

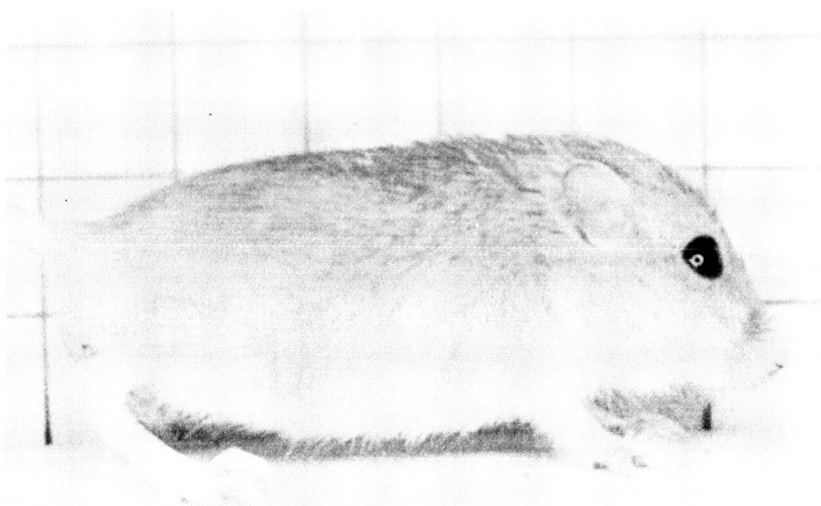


12 days

PLATE I *Perognathus longimembris* development (1-12 days). Grid: 1 cm^2

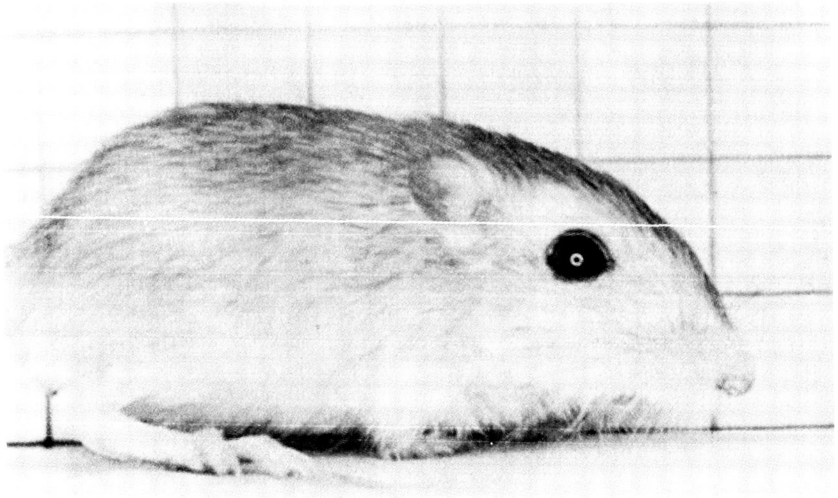


19 days



28 days

PLATE II <u>Perognathus</u> <u>longimembris</u> development (19-28 days). Grid: 1 cm^2



38 days

57 days

PLATE III <u>Perognathus longimembris</u> development (38-57 days). Grid: 1 cm^2

Gestation and Maternal Care

Gestation periods could be definitely established in 31 of the pregnancies. Gestation lasts 22 to 23 days in 74% of these cases. Gestations as short as 21 days and as long as 30 days were recorded. One animal littered at 30 days but cannibalized the litter shortly after parturition.

Spontaneous abortions and subsequent cannibalizations of the young occurred in five of the 48 litters. Several isolated deaths of neonatal mice have been observed but could not be attributed to maternal neglect.

Maternal care was almost excessive and often involved much aimless carrying and shifting of neonates. The impulse to shift young occurred as late as the third week, an age when offspring are almost as large as the dam. Even at this age, the young did not struggle when picked up by the dam; rather, they facilitated the action by raising their legs. Litters were weaned at about 30 days.

Discussion and Conclusions

Laboratory breeding of *Perognathus longimembris* can be induced by proper selection of mating pairs. The key to selecting compatible pairs is routine observation of large numbers of both sexes to ascertain their state of reproductive activity. Then, females in full estrus, that is with open vaginal orifice, are placed with males with enlarged testes, resulting in copulation and conception approximately 20 percent of the time.

It is evident from our data that reproductive cycles in laboratory-maintained pocket mice coincide with the natural breeding season as judged by ecological and field collection data. For example, the high incidence of juveniles in field samples taken in June would suggest that conception occurred in field animals at approximately the same time our laboratory animals were showing a high incidence of estrous cycles.

Obviously, routine observation and selective pairing of large numbers of pocket mice is time consuming and expensive. As suggested in our earlier report, there are several alternate courses open to improve the situation. Much depends upon whether laboratory-conceived and -reared animals will exhibit modified physiological and behavioral patterns.

Our effort is now being directed toward obtaining as much observational data as possible on laboratory-born animals. Particular attention is being paid to estrus in these animals and to the possibility that the polyestrous period can be lengthened and the incidence of estrus increased in a laboratory colony of pocket mice. There is some evidence that ecological conditions shift slightly the time of appearance of natural breeding cycles (1). It is not known whether changes in breeding activity periods can be induced by alterations in the laboratory environment. On the contrary, it appears that pocket mice maintained for long periods in a uniform laboratory environment had a breeding season which coincided with field animals. Nevertheless, as this study continues, environmental and physiological factors will be altered selectively in an attempt to improve the efficiency of laboratory breeding of Perognathus.

REFERENCE

1. Hayden, P., J. J. Gambino and R. G. Lindberg, Reproduction of Perognathus in Captivity. Progress Report NSL 64-29-6, Contract NASw-812. Submitted to NASA, 1 January through 31 March 1965.